IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant	:	de Paoli Ambrosi
Filing date	:	October 3, 2006
Title	:	Formulation for Chemical Peeling
TC/A.U.	:	1619
Examiner	:	**Jyothsna A. Venkat**
Docket No.	:	**5759**
Customer No.	:	26936

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

I, the undersigned Dr. Gianfranco de Paoli Ambrosi, declare as follows:

1. I have a degree in pharmacy from the Università degli Studi di Pavia-Italy and a master in cosmetic science and technologies from the Università degli Studi di Milano- Italy.

2. I am inventor of several patent applications in the field of cosmetics and, in particular, concerning preparations for topical administration.

3. I am also inventor of the present patent application n. 10/557,923 concerning a formulation for chemical peeling. In particular, the present invention concerns composition comprising at least one keratolytic agent and dimethyl isosorbide, wherein said keratolytic agent, alone or as a mixture of two or more keratolytic agents, and said dimethyl isosorbide are each contained in a quantity of between 1% and 99% by weight.

4. On about June 2003, I engaged the University of Catania to perform tests to evaluate the peeling effect as well as the induced cutaneous erythema when using two formulations comprising pyruvic acid and dimethylisosorbide (Pyruvic acid ENERPEEL TECHNOLOGY 50%) and glycolic acid and dimethylisosorbide (Glycolic acid ENERPEEL TECHNOLOGY 50%). The assays have to be performed in comparison with compositions containing pyruvic acid only, thus lacking dimethyl isosorbide. The results of tests performed by the University were reported to me by Dr. Bonina in August 2003 and are here enclosed as Annex 1 and Annex 2, respectively.

5. The evaluation has been conducted by the University of Catania-Dipartimento di Scienze Farmaceutiche, Catania-Italy.

6. A first set of experiments is reported in Annex 1 attached to the present declaration.

7. The experimental conditions applied are detailed in the first three paragraphs, disclosing the number and type of selected candidate volunteers, how skin pigmentation has been induced, the referenced compounds used, the quantity of the tested compounds and the application procedures.

8. In particular, the tested compound is a composition containing a keratolytic agent, pyruvic acid, in a quantity of 50% by weight in the presence of dimethyl isosorbide (ENERPREEL TECHNOLOGY) i.e. a composition of the invention, and the same keratolytitic agent in a quantity of 50% by weight but without dimethyl isosorbide (not buffered aqueous solution).

9. According to the method, on some small skin areas of the volunteers a pigmentation was induced by DHA gel application. Volunteers undergo at least two examinations to assess: i) the peeling effect; ii) the induced erythematogenous effect.

10. The two effects were evaluated through reflectance spectrometry thanks to the Melanin Index (MI) and Erythema Index (E.I.), respectively, as detailed in Annex 1.

11. The results of the assays are summarized in the Results section of the Annex 1.

12. The MI-time curve, i.e. the time interval required by each skin site to obtain an MI value close or equal to the baseline value taken at the beginning of the experiment before application of the DHA gel, a time defined as "Recovery Time" (RT) is an indicator of an increased corneum stratum turnover and therefore of the peeling effect of the tested preparations. ENERPEEL Technology Glycolic acid 50% appears to provide a significant increase in the *stratum corneum* turnover rate, greater than that provided by the corresponding 50% Glycolic acid not buffered aqueous solution, as evident from data reported in Table 2.

13. The Area Under the Curve (AUC) values were calculated from the EI-time curve. The AUC values are proportional to the intensity and duration of the erythema and therefore to the inflammatory effects induced by individual preparations. The ENERPEEL Technology pyruvic acid 50% preparation causes less skin irritation than the 50% pyruvic acid not buffered aqueous solution preparation, as evident from data reported in Table 3.

14. A second set of experiments based on DHA treatment and reflectance spectrometry have been performed as reported in Annex 2 attached to the present declaration.

15. The experimental conditions are the same disclosed in Annex 1 and are detailed in the first three paragraphs of Annex 2.

16. In particular, the tested compound is a composition containing a keratolytic agent, glycolic acid, in a quantity of 50% by weight or 30% by weight in the presence of dimethyl isosorbide

(ENERPREEL Technology 50%, ENERPREEL Technology 30%) i.e. a composition of the invention, and the same keratolytitic agent in a quantity of 50% by weight but without dimethyl isosorbide (not buffered aqueous solution).

17. According to the method, on some small skin areas of the volunteers a pigmentation was induced by DHA gel application. Volunteers undergo at least two examinations to assess: i) the peeling effect; ii) the induced erythematogenous effect.

18. The two effects were evaluated through reflectance spectrometry thanks to the Melanin Index (MI) and Erythema Index (EI), respectively, as detailed in Annex 2.

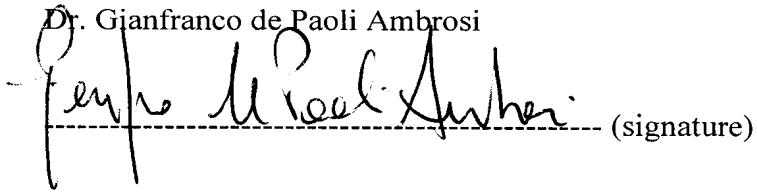
19. The results of the assays are summarized in the Results section of the Annex 2.

20. The MI-time curve, i.e. the time interval required by each skin site to obtain an MI value close or equal to the baseline value taken at the beginning of the experiment before application of the DHA gel, a time defined as "Recovery Time" (RT) is an indicator of an increased corneum stratum turnover and therefore of the peeling effect of the tested preparations. ENERPEEL Technology glycolic acid 50% appears to provide a significant increase in the *stratum corneum* turnover rate, greater than that provided by the corresponding 50% glycolic acid not buffered aqueous solution, as evident from data reported in Table 2. ENERPEEL Technology Glycolic acid 30% preparation is seen to be less active than the ENERPEEL Technology Glycolic acid 50% preparation and the 50% glycolic acid not buffered aqueous solution .

21. The Area Under the Curve (AUC) values were calculated from the E.I.-time curve. The AUC values are inversely proportional to the intensity and duration of the erythema and therefore to the inflammatory effects induced by individual preparations. The ENERPEEL Technology Glycolic acid 50% preparation causes less skin irritation than the 50% Glycolic acid not buffered aqueous solution preparation, as evident from data reported in Table 3.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that the making of wilful false statements or the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patents issued thereon.

(date) Dr. Gianfranco de Paoli Ambrosi



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ANNEX 1

Assessment of a not buffered free water solution of 50% pyruvic acid compared to a 50% Pyruvic acid in Enerpeel Technology.

The aim of this research was to assess the peeling effect induced on skin by two formulations: ENERPEEL Technology pyruvic acid 50% (Pyruvic acid 50% by weight in the presence of dimethyl isosorbide) and 50% pyruvic acid not buffered aqueous solution (Pyruvic acid 50% by weight). The model of dihydroxyacetone (DHA) which induces the *corneum* pigmentation was used in assessing the research. DHA is an active ingredient currently used into cosmetic tanning products that works in conjunction with the *stratum corneum* proteins to create a color change with chromophore characteristics similar to those of melanin. The rate at which skin pigmentation DHA induced vanishes, depends on the "skin turnover". Monitoring the latter with non-invasive technology such as reflectance spectrophotometry (1-7) enables the cosmetic treatment effects assessment on skin turnover (peeling effect). A number of skin sites were first colored with a DHA gel and then administered brief daily applications of preparations ENERPEEL Technology Pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution The *stratum corneum* pigmentation index was then monitored for twenty days by reflectance spectrophotometry (2-8). Through reflectance spectrometry, it is actually possible to monitor the DHA – skin protein bond thanks to the Melanin Index (MI). The MI is easily detectable from the skin absorbtion spectrum. DHA tanned skin, like naturally tanned skin, is characterized by a high level of absorption in the UV range while absorption steadily decreases from 450 nm to 700 nm in the visible spectrum. Moreover, in the 650 nm to 700 nm spectrum ranges, where there are no hemoglobin absorption peaks, DHA pigmentation still has significant absorption. On this premise, the DHA pigmentation intensity factor may therefore be determined using the same MI obtainable from spectra data in the 650 nm to 700 nm ranges from which the quantity of melanin in the skin can be calculated.

$$MI = LIR\ 650 - LIR\ 700 + 0.015,$$

in which LIR is the Logarithm of Inverse Reflectance measured with a reflectance spectrophotometer at 650 nm and 700 nm respectively. The figure of 0.015 is the correction factor and depends on the instrument used. The MI value traces the slope between 650 nm and 700 nm on the graph. Moreover, thanks to reflectance spectrometry, also the rash effect induced by the peeling to the skin, has been evaluated.

Protocol used in assessing the peeling effects of preparations Enerpreel Technology Pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution.

Twelve healthy volunteers (all skin photo-types II and III) were involved in the peeling effect tests of the three formulations and eight skin sites (1 cm2) were established on the central part of each volunteer's forearm. The skin sites where initially the MI baseline value was evaluated have subsequently been colored by a 4% DHA gel application. The applications lasted 90 minutes and were conducted using specific chambers (Hill Top Research Inc., Cincinnati, OH) containing 150 mg of DHA gel. Three to four hours after removing the DHA gel, each skin site showed a growing increase in colour up to the steady state after 12-24 hours. Twenty four hours after removing the DHA gel, each skin site was treated with a Hill Top Chamber application (in twos, over a 20 day period). Each chamber contained a cotton pad soaked in 200 microl of one of the test solutions (ENERPEEL Technology pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution). Two of the DHA coloured test sites were left untreated and were used as control sites. The application time of the ENERPEEL Technology Pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution varied from 3 – 6 minutes depending on the subject's sensitivity to the acid. The chambers were removed after the application (3 – 6 minutes) and the sites subjected to the glycolic acid formulation were then treated with a neutralising solution to counter cutaneous acidity.

Monitoring for each site was carried on by measuring variations in the MI over time for a 20-day period. The skin pigmentation intensity and duration was quantified by calculating the values of the area under the MI-time curve (AUC). The AUC values showed to be proportional to skin pigmentation intensity and duration but inversely proportional to the skin turnover and hence to the skin peeling rate. A low AUC value implies a rapid exchange process and thus faster removal of DHA induced pigmentation. Another significant parameter emerging from the MI-time curve is the time interval required by each skin site to obtain a MI value close or equal to the baseline value taken at the beginning of the experiment before application of the DHA gel. This parameter which we call "Recovery Time" (RT) is a reasonably good indicator of any increase in corneum stratum turnover and therefore of the peeling effect of the tested preparations.

Protocol used in assessing cutaneous erythema induced by formulations ENERPEEL Technology Pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution.

Twelve healthy volunteers (the same subjects as used in the peeling effect assessment) were involved in the induced erythematogenous effect assessment tests. They were informed in advance about the experiment's nature and the procedures to be performed. Volunteers characterized by skin photo-types II to III were selected and their written consent was requested. Six skin sites (1 cm^2) were established on the central part of each volunteer's forearm and these sites were treated in pairs with an application of formulations ENERPEEL Technology Pyruvic acid 50% and 50% Pyruvic acid not buffered aqueous solution. The application time of the preparations ranged (varied) from 8 – 12 minutes depending on the subject's sensitivity to Pyruvic acid. The different application times

in this protocol, compared to the times used in assessing the peeling effects, are justifiable as they were used to: a) produce a more intense form of erythema and better distinguish the inflammatory effects induced by the individual formulations. b) produce a sufficiently intense form of erythema to enable a longer monitoring period (60 hours) without creating undue problems for the subjects. This was possible because this protocol specified a single application, not daily or repeated doses. The preparations were applied using Hill Top Chambers (Hill Top, Cincinnati, OH) and on completion of the applications, the chambers were removed and the skin sites treated with a suitable neutralizing solution. The erythema on each site was monitored by a reflectance spectrophotometer for 60 days (X-Rite mod.968). The instrument was calibrated to meet the National Bureau of Standards blind test requirements using a light source C and an angle of observation of 2°. The spectrophotometer was connected to a PC which SPECTROSTART software proceeded the skin's spectrum reflectance results (from 400 to 700 nm).

Figure 2 shows spectrum reflectance results for the same skin sites before (curve A) and after (curve B) application of the formulation containing Pyruvic acid. As can be seen from curve B, the spectrum reflectance on the skin sites after an application of the formulation shows two absorption bands: a single band close to 400 nm and a double band between 540 and 580 nm which relates to hemoglobin absorption. The index of erythema (EI) over time for each skin site can be calculated from the spectra data obtained using the equation below. This index, proposed by Dawson (8), is an important parameter for quantitatively monitoring skin erythema.

$$I.E. = 100\left[\log\frac{1}{R560} + 1{,}5\left(\log\frac{1}{R540} + \log\frac{1}{R580}\right) - 2\left(\log\frac{1}{R510} + \log\frac{1}{R610}\right)\right]$$

In the equation, the logarithmic values of the reciprocal of reflectance (wavelengths 540, 560 and 580) showing the hemoglobin peaks are totaled and the corresponding wavelength values (510 and 610) where absorption is mainly due to the presence of melanin, are subtracted from them. The EI base values, determined for each site before applying the formulation, were subtracted from the EI values of the same skin sites taken at different times, thus obtaining typical graph curves. The Area Under the Curve (AUC) values were then calculated from the resulting graph. The AUC values are of particular importance in the assessment of erythema as they are proportional to the intensity and duration of the erythema and therefore to the inflammatory effects induced by individual preparations.

Results

The three tested preparations all increase the skin turnover rate and reduce the intensity and duration of DHA induced pigmentation in comparison to the control sample, (see the AUC values in **Table 1**). They also reduce the time required for the skin to return to its original condition (see

"Recovery Time" in **Table 2**). It should be underlined that among the tested formulations, ENERPEEL Technology Pyruvic acid 50% provided a better peeling effect, as can be seen from the lower AUC values and shorter recovery times shown in Tables 1 and 2 ($p < 0.01$) than a conventional preparation (50% Pyruvic acid not buffered aqueous solution).

ENERPEEL Technology pyruvic acid 50% causes less irritation and creates a less intense erythematogenous effect than that provoked by the 50% pyruvic acid not buffered aqueous solution, as highlighted by the AUC values shown in **Table 3** and related to the areas under the EI-time curve that are significantly lower for the ENERPEEL Technology Pyruvic acid 50% preparation than for 50% Pyruvic acid not buffered aqueous solution.

On the basis of the results obtained from this series of experiments, we can therefore affirm that:

1) ENERPEEL Technology pyruvic acid 50% appears to provide a significant increase in the stratum corneum turnover rate, greater than that provided by the corresponding 50% pyruvic acid not buffered aqueous solution. This effect may be attributed to better skin penetration by the pyruvic acid in the Enerpeel Technology compared to that of 50% pyruvic acid not buffered aqueous solution.

2) The ENERPEEL Technology pyruvic acid 50% preparation, despite offering a superior peeling effect, causes less skin irritation than the 50% pyruvic acid not buffered aqueous solution preparation. This last result is very interesting if one bears in mind the number of times subjects may apply preparations before achieving aesthetically satisfactory results.

Bibliography

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FIGURES AND TABLES

Figure 1. Reflectance spectra of pigmented (DHA-treated) skin sites (B) and non-pigmented skin sites (A).

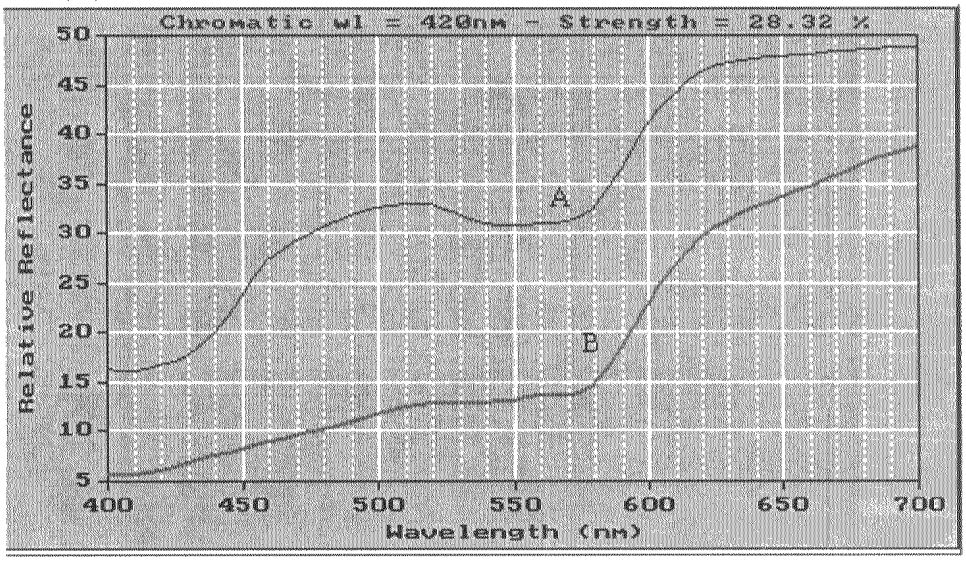

Figure 2. Reflectance spectra of the skin, obtained before (curve a) and after (curve b) exposure to UVB.

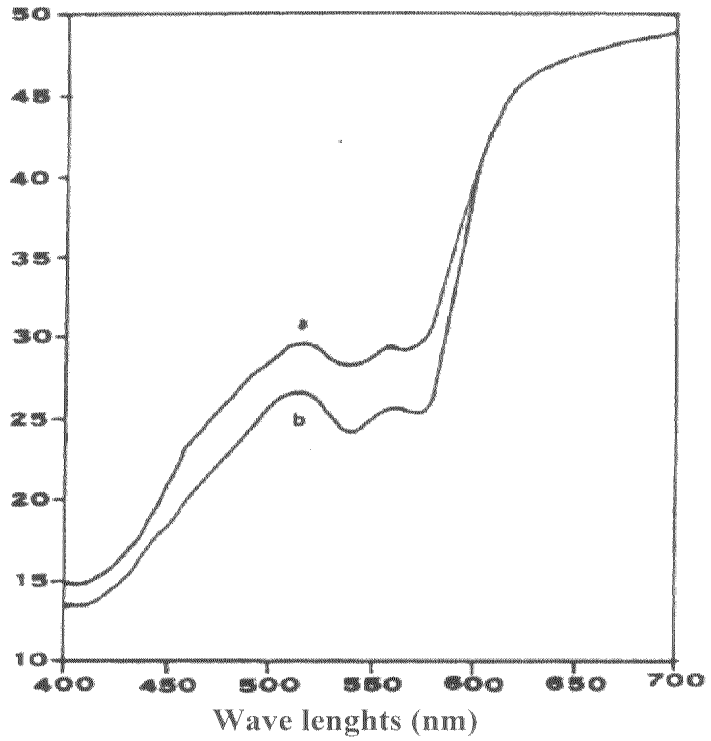

Table 1 Variations in the AUC of MI-time curves obtained for individual subjects during the monitoring of DHA induced pigmentation and subsequent treatment with: 50% pyruvic acid not buffered aqueous solution and ENERPEEL Technology pyruvic acid 50%.

Patients	Control	Pyruvic acid Enerpeel	Pyruvic acid aqueous
A	125.2	58.4	72.3
B	135.8	63.7	78.4
C	141.7	72.5	87.1
D	112.6	64.3	58.6
E	148.5	73.8	84.7
F	140.3	72.8	91.3
G	118.3	53.7	64.2
H	129.4	80.6	78.4
I	133.2	55.4	110.6
L	158.1	60.9	82.6
M	110.3	96.4	81.8
N	131.7	57.3	91.5
Average	**132.0**	**67.5***	**81.8***
S.D.	14.2	12.4	11.5

* $p < 0.05$ (t-test)

Table 2 Variations in "Recovery Times" obtained for individual subjects during the monitoring of DHA induced pigmentation and subsequent treatment with: 50% pyruvic acid not buffered aqueous solution; ENERPEEL Technology pyruvic acid 50%.

Patients	Control	Pyruvic acid Enerpeel	Pyruvic acid aqueous
A	20	10.1	11.5
B	19.5	9.5	12.3
C	20.5	11.4	12.6
D	18.5	11.3	10.8
E	19	10.3	11.5
F	20	11.1	11.7
G	19.5	10.3	10.9
H	18	11.4	12.5
I	20.5	11.0	12.1
L	20	10.3	11.6
M	19.5	9.1	10.5
N	18.5	10.5	12.3
Average	**19.46**	**10.5***	**11.7***
S.D.	0.81	0.74	0.69

*$p < 0.01$ (t-test)

Table 3 AUC values of the MI-time curve obtained for skin sites treated with ENERPEEL Technology pyruvic acid 50% and with the 50% pyruvic acid not buffered aqueous solution.

Patients	Control	Pyruvic acid Enerpeel	Pyruvic acid aqueous
A	20	538.4	780.5
B	19.5	495.3	698.3
C	20.5	557.1	635.8
D	18.5	358.6	769.4
E	19	542.3	538.6
F	20	538.4	658.4
G	19.5	426.8	798.3
H	18	579.6	638.7
I	20.5	445.6	726.5
L	20	573.2	668.1
M	19.5	526.1	647.3
N	18.5	583.4	749.5
Average	19.46	513.7*	692.4*
S.D.	0.81	69.6	75.6

*$p < 0.01$ (t-test)

ANNEX 2

Assessment of the glycolic acid based formulations GLY-A and GLY-B (General Topics) and GLY-C in skin peeling and induced cutaneous erythema.

The aim of this research was to assess the effects on skin peeling induced by two General Topics formulations (ENERPEEL Technology Glycolic acid 50% and – ENERPEEL Technology Glycolic acid 30%, i.e. composition comprising Glycolic acid at the 50% or 30% by weight, respectively, and dimethyl isosorbide) and by a conventional formulation (50% Glycolic acid not buffered aqueous solution).

The model of dihydroxyacetone (DHA) induced *stratum corneum* pigmentation was used in assessing the research. DHA is an active ingredient in cosmetic tanning products that works in conjunction with proteins in the stratum corneum to create a colour change with chromophore characteristics similar to those of melanin.

The rate at which DHA induced skin pigmentation occurs depends on the "skin turnover". Monitoring the latter with non-invasive technology such as reflectance spectrophotometry (1-7) enables assessment of the effects of cosmetic treatments on skin turnover (peeling effect).

A number of skin sites were first coloured with a DHA gel and then administered brief daily applications of preparations ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% glycolic acid not buffered aqueous solution The *stratum corneum* pigmentation index was then monitored for twenty days by reflectance spectrophotometry (2-8).

Skin pigmentation by the DHA-protein agent can be monitored by the melanin index (MI) using reflectance spectrophotometry. This index is easily obtained from the skin reflectance spectrum. DHA tanned skin, like naturally tanned skin, is characterized by a high level of absorption in the UV range while absorption steadily decreases from 450 nm to 700 nm in the visible spectrum. In the 650 nm to 700 nm spectrum ranges where there are no hæmoglobin absorption peaks, DHA pigmentation still has significant absorption. On this premise, the DHA pigmentation intensity factor may therefore be determined using the same melanin index obtainable from spectra data in the 650 nm to 700 nm ranges from which the quantity of melanin in the skin can be calculated.

$$MI = LIR\ 650 - LIR\ 700 + 0.015,$$

in which LIR is the Logarithm of Inverse Reflectance measured with a reflectance spectrophotometer at 650 nm and 700 nm respectively. The figure of 0.015 is the correction factor and depends on the instrument used. The MI (Melanin Index) value traces the slope between 650 nm and 700 nm on the graph.

Protocol used in assessing the peeling effects of preparations ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% Glycolic acid not buffered aqueous solution.

Twelve healthy volunteers (all skin photo-types II and III) were involved in the peeling effect tests of the three formulations and eight skin sites (1 cm^2) were established on the central part of each volunteer's forearm. An initial MI baseline value for each of these sites was taken and they were then coloured by an application of a 4% DHA gel.

The applications lasted 90 minutes and were conducted using specific chambers (Hill Top Research Inc., Cincinnati, OH) containing 150 mg of DHA gel

Three to four hours after removal of the gel, the sites displayed a change in colour that intensified over time and became stable in the following 12 – 24 hours.

Twenty-four hours after removal of the DHA gel, each skin site was treated with a Hill Top Chamber application (in twos, over a 20 day period). Each chamber contained a cotton pad soaked in 200 microl of one of the test solutions (ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% glycolic acid not buffered aqueous solution). Two of the DHA coloured test sites were left untreated and were used as control sites.

The application time of the Glycolic acid preparations (ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% Glycolic acid not buffered aqueous solution) varied from 3 – 6 minutes depending on the subject's sensitivity to the acid. The chambers were removed after the application (3 – 6 minutes) and the sites subjected to the Glycolic acid formulation were then treated with a neutralising solution to counter cutaneous acidity.

Monitoring of DHA-induced skin pigmentation for each site was carried out by measuring variations in the melanin index over time for a 20-day period (see **Figure 3**). The intensity and duration of skin pigmentation was quantified by calculating the values of the area under the MI-time curve (AUC). The AUC values were proportional to skin pigmentation intensity and duration but inversely proportional to the skin turnover and hence skin peeling rate. A low AUC value implies a rapid exchange process and thus faster removal of DHA induced pigmentation.

Another significant parameter from the MI-time curve is the time interval required by each skin site to obtain an MI value close or equal to the baseline value taken at the beginning of the experiment before application of the DHA gel. This parameter which we call "Recovery Time" (RT) is a reasonably good indicator of any increase in corneum stratum turnover and therefore of the peeling effect of the preparations tested

Protocol used in assessing cutaneous erythema induced by formulations ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% glycolic acid not buffered aqueous solution .

Twelve healthy volunteers (the same subjects as used in the peeling effect assessment) were involved in the induced erythematogenous effect assessment tests. They were informed in advance about the nature of the experiment and the procedures to be performed. Volunteers from skin photo-types II and III were selected and their written consent was requested.

Six skin sites (1 cm^2) were established on the central part of each volunteer's forearm and sites were treated in pairs with an application of formulations ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30% and 50% Glycolic acid not buffered aqueous solution. The application time of the preparations varied from 8 – 12 minutes depending on the subject's sensitivity to glycolic acid. The different application times in this protocol in comparison to times used in assessing the peeling effects are justifiable as they were used to:

a) produce a more intense form of erythema and better distinguish the inflammatory effects induced by the individual formulations.

b) produce a sufficiently intense form of erythema to enable a longer monitoring period (60 hours) without creating undue problems for the subjects. This was possible because this protocol specified a single application, not daily or repeated doses.

The preparations were applied using Hill Top Chambers (Hill Top, Cincinnati, OH) and on completion of the applications, the chambers were removed and the skin sites treated with a suitable neutralising solution.

The erythema on each site was monitored for 60 days by a reflectance spectrophotometer (X-Rite mod.968). The instrument was calibrated to meet the National Bureau of Standards blind test requirements using a light source C and an angle of observation of 2°. The spectrophotometer was connected to a PC and the skin spectrum reflectance results from the 400 nm – 700 nm ranges were processed using Spectrostart software.

Figure 1 shows spectrum reflectance results for the same skin sites before (curve a) and after (curve b) application of the formulation containing glycolic acid. As can be seen from curve B, the spectrum reflectance on the skin sites after an application of the formulation shows two absorption bands: a single band close to 400 nm and a double band between 540 and 580 nm which relates to hæmoglobin absorption.

The index of erythema (EI) over time for each skin site can be calculated from the spectra data obtained using the equation below. This index, proposed by Dawson (8), is an important parameter for quantitatively monitoring skin erythema.

In the equation,

$$I.E. = 100\left[\log\frac{1}{R560} + 1,5\left(\log\frac{1}{R540} + \log\frac{1}{R580}\right) - 2\left(\log\frac{1}{R510} + \log\frac{1}{R610}\right)\right]$$

the logarithmic values of the reciprocal of reflectance (wavelengths 540, 560 and 580) showing the hæmoglobin peaks are totalled and the corresponding wavelength values (510 and 610) where absorption is mainly due to the presence of melanin, are subtracted from them.

The EI base values, determined for each site before application of the formulation were subtracted from the EI values of the same skin sites taken at different times, thus obtaining typical graph curves (EI- time see **Figure 3**). The Area Under the Curve (AUC) values were then calculated from the resulting graph. The AUC values are of particular importance in the assessment of erythema as they are proportional to the intensity and duration of the erythema and therefore to the inflammatory effects induced by individual preparations.

Results

As can be seen from the MI-time curves (see **Figure 2**), in comparison to the control sample, the three tested preparations all increase the skin turnover rate and reduce the intensity and duration of DHA induced pigmentation (see the AUC values in **Table 1**). They also reduce the time required for the skin to return to its original condition (see "Recovery Time" in **Table 2**).

It should be underlined that among the tested formulations, ENERPEEL Technology Glycolic acid 50% provided a better peeling effect, as can be seen from the lower AUC values and shorter recovery times shown in **Tables 1 and 2** ($p < 0.01$) than a conventional preparation (50% Glycolic acid not buffered aqueous solution).

From the data shown in **Tables 1 and 2**, ENERPEEL Technology Glycolic acid 30% preparation is seen to be less active than the ENERPEEL Technology Glycolic acid 50% preparation and the 50% Glycolic acid not buffered aqueous solution .

Figure 3 shows the graph curves obtained from monitoring EI variations over time after applying the three preparations to the skin.

As can be seen, the ENERPEEL Technology Glycolic acid 50% and ENERPEEL Technology Glycolic acid 30% cause less irritation and create a less intense erythematogenous effect than that provoked by the 50% glycolic acid not buffered aqueous solution. The results of this experiment are further confirmed by the AUC values shown in **Table 3** and relate to the areas under the E.I.-time curve that are significantly lower for the ENERPEEL Technology Glycolic acid 50%

and ENERPEEL Technology Glycolic acid 30% preparations than for 50% Glycolic acid not buffered aqueous solution.

On the basis of the results obtained from this series of experiments, we can therefore affirm that:

1) both ENERPEEL Technology Glycolic acid 50% and ENERPEEL Technology Glycolic acid 30%, but especially ENERPEEL Technology Glycolic acid 50%, appear to provide a significant increase in the *stratum corneum* turnover rate. With ENERPEEL Technology Glycolic acid 50%, the effect is greater than that provided by the corresponding GLY-C aqueous solution. This effect may be attributed to better skin penetration by the cholic acid content of the GLY-A preparation compared to that of 50% glycolic acid not buffered aqueous solution.

2) the ENERPEEL Technology Glycolic acid 50% preparation despite offering a superior peeling effect causes less skin irritation than the 50% Glycolic acid not buffered aqueous solution preparation. This last result is very interesting if one bears in mind the number of times subjects may apply preparations before achieving results which they find aesthetically satisfactory.

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FIGURES AND TABLES

Figure 1. Reflectance spectra of the skin, obtained before (curve a) and after (curve b) exposure to UVB.

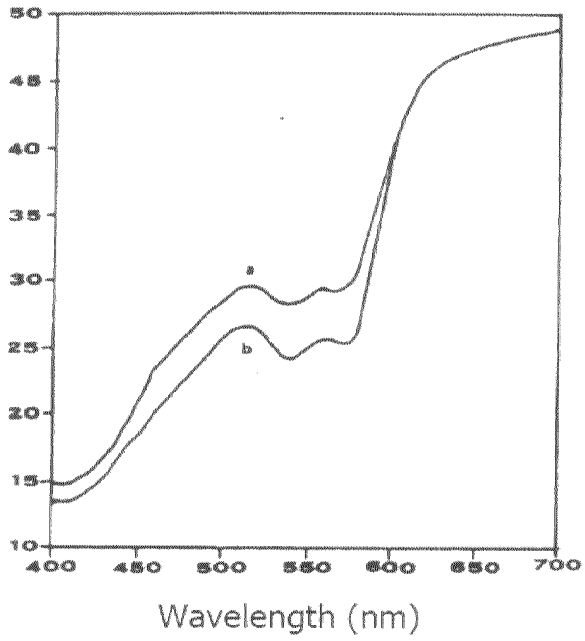

Figure 2. Variations in the MI-time curves obtained for individual subjects during the monitoring of DHA induced pigmentation and subsequent treatment with: 50% glycolic acid not buffered aqueous solution ENERPEEL Technology Glycolic acid 50% and ENERPEEL Technology Glycolic acid 30%.

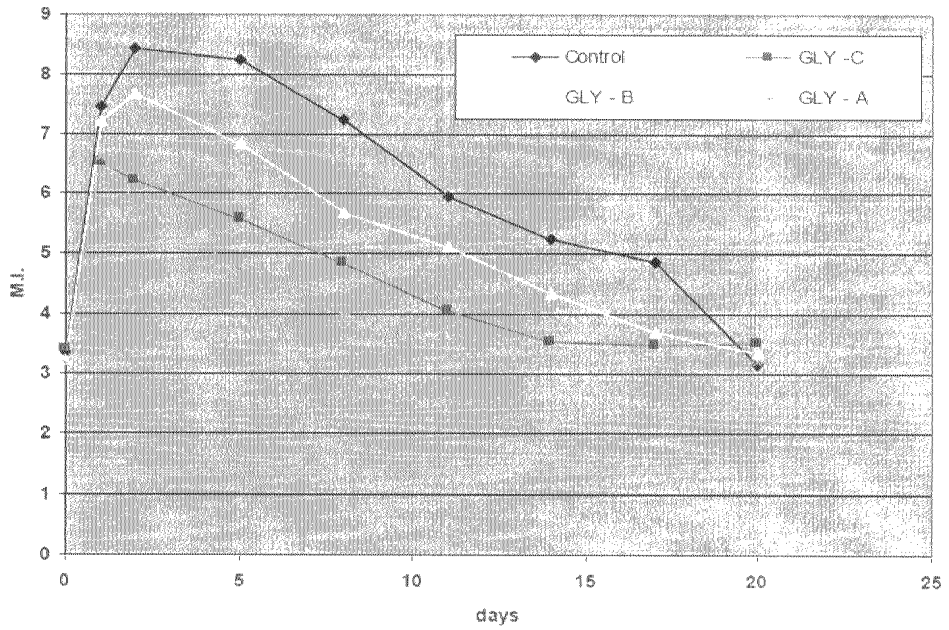

Table 1 Variations in the AUC of MI-time curves obtained for individual subjects during the monitoring of DHA induced pigmentation and subsequent treatment with: 50% glycolic acid not buffered aqueous solution, ENERPEEL Technology Glycolic acid 50% and ENERPEEL Technology Glycolic acid 30%.

SUBJECT	CONTROL	ENERPEEL 50%	ENERPEEL 30%	Glycolic acid 50%
A	125.2	90.3	106.2	80.1
B	135.8	71.7	124.2	85.4
C	141.7	84.2	116.3	93.7
D	112.6	75.8	98.4	74.2
E	148.5	87.5	121.3	108.3
F	140.3	80.6	110.8	102.8
G	118.3	68.3	97.5	71.3
H	129.4	74.3	92.4	85.4
I	133.2	62.8	122.9	113.2
L	158.1	85.4	117.5	84.3
M	110.3	105.7	95.9	95.8
N	131.7	70.8	110.3	102.5
MEAN VALUE	132.0	79.7*	109.4	91.4*
S.D.	14.2	11.7	11.3	13.4

* $p < 0.05$ (t-test)

Table 2 Variations in "Recovery Times" obtained for individual subjects during the monitoring of DHA induced pigmentation and subsequent treatment with: 50% glycolic acid not buffered aqueous solution; ENERPEEL Technology Glycolic acid 50% , ENERPEEL Technology Glycolic acid 30%.

SUBJECT	CONTROL	ENERPEEL 50%	ENERPEEL 30%	Glycolic acid 50%
A	20	11	17	14
B	19.5	11.5	16.5	13.5
C	20.5	12.5	16	14.5
D	18.5	12	17.5	12.5
E	19	11.5	16.5	13.5
F	20	12	17	14.5
G	19.5	11	16	14
H	18	12.5	15.5	13.5
I	20.5	10.5	16.5	12.5
L	20	11.5	17	13.5
M	19.5	10	18	12.5
N	18.5	11.5	17.5	13
MEAN VALUE	19.46	11.46*	16.75	13.33*
S.D.	0.81	0.75	0.72	0.65

- $p < 0.01$ (t-test)

Figure 3. Variations in E.I. over time obtained for sites treated with ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30%and with the 50% glycolic acid not buffered aqueous solution.

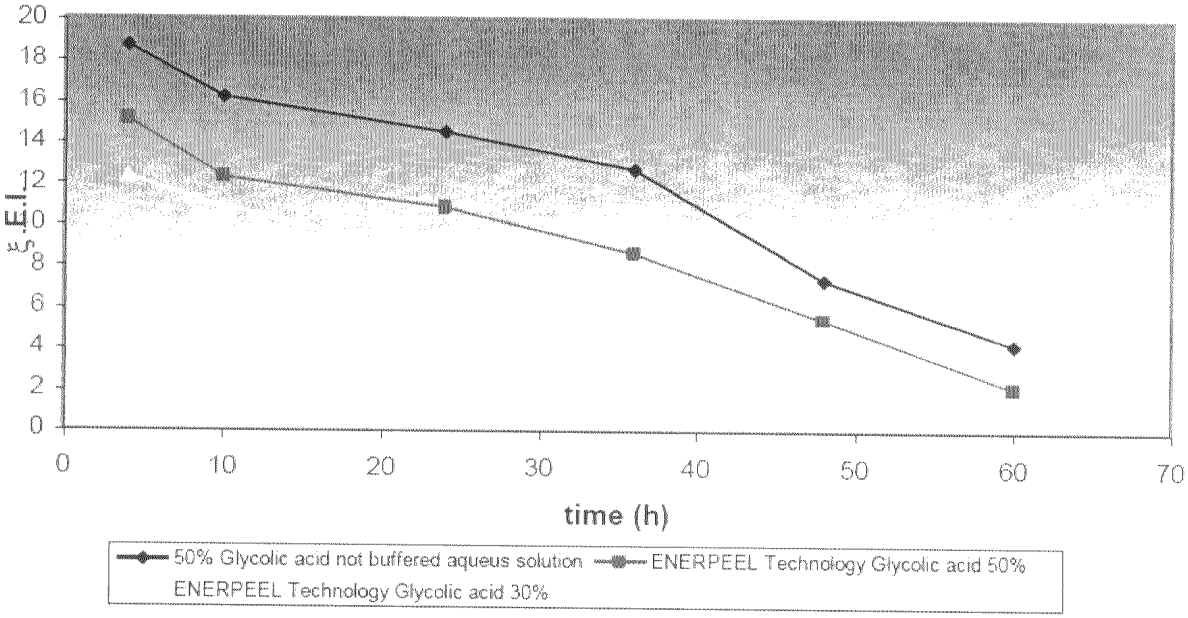

Table 3 AUC values of the MI-time curve obtained for skin sites treated with ENERPEEL Technology Glycolic acid 50%, ENERPEEL Technology Glycolic acid 30%and with the 50% Glycolic acid not buffered aqueous solution.

SUBJECT	ENERPEEL 50%	ENERPEEL 30%	Glycolic acid 50%
A	489.3	392.1	671.8
B	425.8	421.6	623.4
C	561.4	325.1	587.2
D	397.6	357.9	634.7
E	452.5	423.8	704.2
F	489.2	370.4	514.5
G	574.8	512.6	724.6
H	376.4	472.9	536.3
I	415.8	413.5	628.4
L	493.7	342.8	600.9
M	543.6	286.3	571.6
N	565.5	449.1	660.5
MEAN VALUE	482.1*	397.3*	621.5
S.D.	69.2	64.7	63.6

* $p < 0.01$ (t-test)